

SEQUENCE LISTING

<110> Jung, Rudolf  
Beach, Larry R.  
Dress, Virginia M.  
Rao, A. Gururaj  
Ranch, Jerome P.  
Ertl, David S.  
Higgins, Regina K.



<120> Alteration of Amino Acid Compositions in  
Seeds

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<150> US 09/020,716

<151> 1998-02-09

<160> 22

<170> FastSEQ for Windows Version 3.0

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<212> DNA

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<223> pBSKP vector with native alpha hordothionin  
sequence from Hordeum vulgare located from  
nucleotide 3361 to nucleotide 2947.

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 nucleotide 3271 to nucleotide 1834.

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 nucleotide 1757 in *Zea mays* expression vector.  
*Zea mays* promoter from nucleotide 43 to nucleotide  
 1342.

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<211> 5392

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<223> Gene from Glycine max from nucleotide 2199 to nucleotide 2675 in Zea mays expression vector. Zea mays promoter from nucleotide 676 to nucleotide 2198.

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<210> 7

<211> 5173

<212> DNA

<213> Artificial Sequence

<220>

<223> Gene from *Hordeum vulgare* from nucleotide 2199 to nucleotide 2450 in a *Zea mays* expression vector.

*Zea mays* promoter from nucleotide 676 to nucleotide 2198.

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<211> 54  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Primer designed based upon the alpha hordothionin sequence from Hordeum vulgare to amplify the gene and to introduce a NcoI site at the start (ATG) codon and a BamHI site after the stop codon of the thionin coding sequence to facilitate cloning.  
  
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 <210> 9  
 <211> 55  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Primer designed based upon the alpha hordothionin sequence of Hordeum vulgare to amplify the gene and to introduce a NcoI site at the start (ATG) codon and a BamHI site after the stop codon of the thionin coding sequence to facilitate cloning.  
  
 <400> 9  
 cctcacatcc cttagtgcct aagttcgacg tcgggccctc tagtcgacgg atcca 55  
  
 <210> 10  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Primer designed for single stranded DNA site-directed mutagenesis to introduce into the native Hordeum vulgare alpha hordothionin gene 12 codons for lysine, based on the peptide structure of hordothionin 12.  
  
 <400> 10  
 agcggaaaaat gcccgaaagg cttccccaaa ttggc 35  
  
 <210> 11  
 <211> 45  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Primer designed for single stranded DNA site-directed mutagenesis to introduce into the native Hordeum vulgare alpha hordothionin gene 12 codons for lysine, based on the peptide structure of hordothionin 12.  
  
 <400> 11  
 tgcgcaggcg tctgcaagtg taagctgact agtagcgga aatgc 45  
  
 <210> 12  
 <211> 50

<212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer designed for single stranded DNA  
 site-directed mutagenesis to introduce into the  
 native Hordeum vulgare alpha hordothionin gene 12  
 codons for lysine, based on the peptide structure  
 of hordothionin 12.

<400> 12  
 tacaaccttt gcaaagtcaa aggcgccaag aagctttgcg caggcgtctg 50

<210> 13  
 <211> 50  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer designed for single stranded DNA  
 site-directed mutagenesis to introduce into the  
 native Hordeum vulgare alpha hordothionin gene 12  
 codons for lysine, based on the peptide structure  
 of hordothionin 12.

<400> 13  
 gcaagagttg ctgcaagagt accctgggaa ggaagtgcta caacctttgc 50

<210> 14  
 <211> 609  
 <212> DNA  
 <213> Pisum sativum

<220>  
 <221> CDS  
 <222> (18)...(410)

<400> 14  
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 Met Ala Ser Val Lys Leu Ala Ser Leu Met Val  
 1 5 10

ttg ttt gcc aca tta ggt atg ttc ctg aca aaa aac gta gga gca gca 98  
 Leu Phe Ala Thr Leu Gly Met Phe Leu Thr Lys Asn Val Gly Ala Ala  
 15 20 25

agc tgc aat ggg gtt tgt tct cca ttt gag atg cca cca tgt ggc tct 146  
 Ser Cys Asn Gly Val Cys Ser Pro Phe Glu Met Pro Pro Cys Gly Ser  
 30 35 40

tca gcc tgt cga tgt atc cct gtt ggt cta gtt gtt ggt tac tgc aga 194  
 Ser Ala Cys Arg Cys Ile Pro Val Gly Leu Val Val Gly Tyr Cys Arg  
 45 50 55

cat cca tct gga gtt ttc ttg agg acg aat gat gaa cac cct aac tta 242  
 His Pro Ser Gly Val Phe Leu Arg Thr Asn Asp Glu His Pro Asn Leu  
 60 65 70 75

tgt gag tct gat gcc gat tgt agg aag aaa gga agt ggt aac ttt tgc 290  
 Cys Glu Ser Asp Ala Asp Cys Arg Lys Lys Gly Ser Gly Asn Phe Cys  
 80 85 90



gcc tct gca atg cag atg ccc tgc ccc tgc gcg ggg ctg cag ggc ttg	332
Ala Ser Ala Met Gln Met Pro Cys Pro Cys Ala Gly Leu Gln Gly Leu	
20 25 30	
tac ggc gct ggc gcc ggc ctg acg acg atg atg ggc gcc ggc ggg ctg	380
Tyr Gly Ala Gly Ala Gly Leu Thr Thr Met Met Gly Ala Gly Gly Leu	
35 40 45	
tac ccc tac gcg gag tac ctg agg cag ccg cag tgc agc ccg ctg gcg	428
Tyr Pro Tyr Ala Glu Tyr Leu Arg Gln Pro Gln Cys Ser Pro Leu Ala	
50 55 60 65	
gcg gcg ccc tac tac gcc ggg tgt ggg cag acg agc gcc atg tac cag	476
Ala Ala Pro Tyr Tyr Ala Gly Cys Gly Gln Thr Ser Ala Met Tyr Gln	
70 75 80	
ccg ctc cgg caa cag tgc tgc cag cag cag atg agg atg atg gac gtg	524
Pro Leu Arg Gln Gln Cys Cys Gln Gln Gln Met Arg Met Met Asp Val	
85 90 95	
cag tcc gtc gcg cag cag ctg cag atg atg atg cag ctt gag cgt gcc	572
Gln Ser Val Ala Gln Gln Leu Gln Met Met Met Gln Leu Glu Arg Ala	
100 105 110	
gct gcc gcc agc agc agc ctg tac gag cca gct ctg atg cag cag cag	620
Ala Ala Ala Ser Ser Ser Leu Tyr Glu Pro Ala Leu Met Gln Gln Gln	
115 120 125	
cag cag ctg ctg gca gtc cag ggt ctc aac ccc atg gcc atg atg atg	668
Gln Gln Leu Leu Ala Val Gln Gly Leu Asn Pro Met Ala Met Met Met	
130 135 140 145	
gcg cag aac atg ccg gcc atg ggt gga ctc tac cag tac cag tac cag	716
Ala Gln Asn Met Pro Ala Met Gly Gly Leu Tyr Gln Tyr Gln Tyr Gln	
150 155 160	
ctg ccc agc tac cgc acc aac ccc tgt ggc gtc tcc gct gcc att ccg	764
Leu Pro Ser Tyr Arg Thr Asn Pro Cys Gly Val Ser Ala Ala Ile Pro	
165 170 175	
ccc tac tac tga ttcatgatat ttgggaaatc tcctctatcc atccctctct	816
Pro Tyr Tyr *	
180	
atctatatat gtaataatgc agtaagacga cacacattat catgtgtggt atgaccaata	876
atatatgcat cataataaag ttttggtttt aaagaattat cggacgcttg atatctatga	936
tgctggataa atcaaaaactt ctcatataaa ttgtaaatat ttcaaaatct ctatttaggc	996
tccaatggag agcatatggg tagagtagta tatatgcttg aaataactaac aactagcaaa	1056
gtgcgggcac gttgctacat gctcatttat gctcgagcat ggagtataaa acataaagat	1116
atatatgttc cattggcctg gtaaacgctg gatataggtt taaagccaac aactcatggt	1176
tcgaatcccc atttatatat aatccataat tttagcgctt tttaccattt aaattttgga	1236
gtaa	1240

<210> 17  
 <211> 180  
 <212> PRT  
 <213> Zea mays

<400> 17  
 Met Lys Met Val Ile Val Leu Val Val Cys Leu Ala Leu Ser Ala Ala  
 1 5 10 15  
 Cys Ala Ser Ala Met Gln Met Pro Cys Pro Cys Ala Gly Leu Gln Gly



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35	40	45
Leu Tyr Pro Tyr Ala Glu Tyr	Leu Arg Gln Pro Gln Cys Ser Pro Leu	
50	55	60
Ala Ala Ala Pro Tyr Tyr Ala Gly	Cys Gly Gln Thr Ser Ala Met Tyr	
65	70	75
Gln Pro Leu Arg Gln Gln Cys Cys	Gln Gln Met Arg Met Met Asp	
85	90	95
Val Gln Ser Val Ala Gln Gln Leu	Gln Met Met Met Gln Leu Glu Arg	
100	105	110
Ala Ala Ala Ala Ser Ser Ser Leu	Tyr Glu Pro Ala Leu Met Gln Gln	
115	120	125
Gln Gln Gln Leu Leu Ala Val Gln	Gly Leu Asn Pro Met Ala Met Met	
130	135	140
Met Ala Gln Asn Met Pro Ala Met	Gly Gly Leu Tyr Gln Tyr Gln Tyr	
145	150	155
Gln Leu Pro Ser Tyr Arg Thr Asn	Pro Cys Gly Val Ser Ala Ala Ile	
165	170	175
Pro Pro Tyr Tyr		
180		

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 <212> DNA  
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<220>  
 <221> CDS  
 <222> (1137)...(1589)

<400> 18

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catgtgcgca	atgacatacc	atcatatttg	atatcataaa	aataaattta	ttatcaaagt	240
aaacatatag	ttcatatatc	agatattaaa	gtgataagaa	caaataattac	attttatctt	300
atataaaatg	acgaaaaagg	tacgagttga	aaaggagtcc	aaccctttt	ttatagcttg	360
ttcggttget	tggtctcttc	ggctagcgag	gtggtagaat	gtgagagtgt	tgcgcgtgga	420
ttcccgctcg	agtgttctta	ggtgatttct	cacggcccat	ctgtgatata	gcgactcata	480
tgtggtgtaa	tagcccatg	ggagaagggg	agagatatag	atctacgtga	tttgacgtg	540
atgcacgacg	aacgaaactg	gtggtttaaa	gtagtagagg	tttgtcatta	gaggtgtaaa	600
tggtacatat	attatccgtt	catattcgaa	tttgatccgt	ataagagggc	taagatctaa	660
tccgtataca	agtccaagta	ttaagtatcc	gatccatata	ggatctttat	ccgtatccgt	720
atactcaaaa	tttgatgttt	aagattttta	tatatattta	aactttatag	gaactcgata	780
atatttgtat	ctgatttgaa	ttatgaaaac	aaatatggaa	cgattaattt	cagtctatat	840
ccgttccgat	atttgtcatg	ctttgtctaa	aataccttta	caaggcatct	tgtgcagatt	900
atatattaat	ctgaaatcag	ttagagaagc	ctacaaattt	gaccaaatgc	cgagtcatcc	960
ggcttatccc	ctttccaact	ttcagttctg	caagcgccag	aaatcgtttt	tcactctacat	1020
tgtctttgtt	gcctgcatac	atctataaat	aggacctgct	agatcaatcg	cagtcctatcg	1080
gcctcagtcg	cacatatcta	ctatactata	ctctaggaag	caaggacacc	accgcc atg	1139
				Met		
				1		

gca gcc aag atg ctt gca ttg ttc gct ctc cta gct ctt tgt gca agc	1187
Ala Ala Lys Met Leu Ala Leu Phe Ala Leu Leu Ala Leu Cys Ala Ser	
5 10 15	

gcc act agt gcg acc cat att cca ggg cac ttg cca cca gtc atg cca	1235
Ala Thr Ser Ala Thr His Ile Pro Gly His Leu Pro Pro Val Met Pro	
20 25 30	

ttg ggt acc atg aac cca tgc atg cag tac tgc atg atg caa cag ggg Leu Gly Thr Met Asn Pro Cys Met Gln Tyr Cys Met Met Gln Gln Gly 35 40 45	1283
ctt gcc agc ttg atg gcg tgt ccg tcc ctg atg ctg cag caa ctg ttg Leu Ala Ser Leu Met Ala Cys Pro Ser Leu Met Leu Gln Gln Leu Leu 50 55 60 65	1331
gcc tta ccg ctt cag acg atg cca gtg atg atg cca cag atg atg acg Ala Leu Pro Leu Gln Thr Met Pro Val Met Met Pro Gln Met Met Thr 70 75 80	1379
cct aac atg atg tca cca ttg atg atg ccg agc atg atg tca cca atg Pro Asn Met Met Ser Pro Leu Met Met Pro Ser Met Met Ser Pro Met 85 90 95	1427
gtc ttg ccg agc atg atg tgc caa ata atg atg cca caa tgt cac tgc Val Leu Pro Ser Met Met Ser Gln Ile Met Met Pro Gln Cys His Cys 100 105 110	1475
gac gcc gtc tgc cag att atg ctg caa cag cag tta cca ttc atg ttc Asp Ala Val Ser Gln Ile Met Leu Gln Gln Gln Leu Pro Phe Met Phe 115 120 125	1523
aac cca atg gcc atg acg att cca ccc atg ttc tta cag caa ccc ttt Asn Pro Met Ala Met Thr Ile Pro Pro Met Phe Leu Gln Gln Pro Phe 130 135 140 145	1571
gtt ggt gct gca ttc tag atagaaatat ttgtgttgta tcgaataatg Val Gly Ala Ala Phe * 150	1619
agttgacatg ccacgcgtg tgactcatta ttaacaataa aacaagtttc ctcttattat ctttttatat ctctccctat ccattttttgc aaagccatt atcctttact ccctaagtcc caatatattt tagaccttaa attgtatgtc tatattcaaa agaatgacaa taaatctaga catatatata aaacacatac attaatgatt gtatgaatct attaaaatgc taaaacgact aatattatgg gacggaggga gtactttatt agtagattac attgttattt tctctattcc aaatataagt ctgggttttc aatcaatcaa tatatattac catgtccaaa cattttgaat tatatatcta ggtgcagcat ccgtgcacga tcgtaaaaga agcagtcacg gtgttggtcc caaaaaactaa tcgtccgttg tcggtcacct ataaagattc atgaagagaa ccaaaataag gcaatataat taatgtaata tgactcctcc ttttgaatta cttaggaata acataagcaa acaaaaaaag gagaagatca aggtaaataa aggcattttg tgagaaaaca tggaagcata agaatgcata agtaaatgatt tgtgtctctt tatatttttt ttattcacgt gaatttacat agataccatc ggatgttcga tggtaataca atgatgcctt agctccgaga gcttcgaatg atgagcgatt taaaaatact cctatcaatt gttcgaaagt tctttgtctc atgcatgggc aatgtacctc tatttatagg gacggtgcga cgtacaaatt tgtataaaat tatattttta ttcccaaata ctatgcatat gtgtcgggga ccataattag ggtaccctc aaggctccta attctcagct ggtaacccca tcagcataaa gctgcaaagg cct	1679 1739 1799 1859 1919 1979 2039 2099 2159 2219 2279 2339 2399 2459 2519 2562
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<213> Zea mays	
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50		55		60											
Leu	Ala	Leu	Pro	Leu	Gln	Thr	Met	Pro	Val	Met	Met	Pro	Gln	Met	Met
65		70		75		80									
Thr	Pro	Asn	Met	Met	Ser	Pro	Leu	Met	Met	Pro	Ser	Met	Met	Ser	Pro
		85		90		95									
Met	Val	Leu	Pro	Ser	Met	Met	Ser	Gln	Ile	Met	Met	Pro	Gln	Cys	His
		100		105		110									
Cys	Asp	Ala	Val	Ser	Gln	Ile	Met	Leu	Gln	Gln	Gln	Leu	Pro	Phe	Met
		115		120		125									
Phe	Asn	Pro	Met	Ala	Met	Thr	Ile	Pro	Pro	Met	Phe	Leu	Gln	Gln	Pro
		130		135		140									
Phe	Val	Gly	Ala	Ala	Phe										
145				150											

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<220>  
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Ala Tyr Thr Ser Lys Ile Phe Ala Leu Phe Ala Leu Ile Ala Leu Ser	
5 10 15	
gca agt gcc act act gca atc acc act atg cag tat ttc cca cca aca	152
Ala Ser Ala Thr Thr Ala Ile Thr Thr Met Gln Tyr Phe Pro Pro Thr	
20 25 30	
tta gcc atg ggc acc atg gat ccg tgt agg cag tac atg atg caa acg	200
Leu Ala Met Gly Thr Met Asp Pro Cys Arg Gln Tyr Met Met Gln Thr	
35 40 45 50	
ttg ggc atg ggt agc tcc aca gcc atg ttc atg tcg cag cca atg gcg	248
Leu Gly Met Gly Ser Ser Thr Ala Met Phe Met Ser Gln Pro Met Ala	
55 60 65	
ctc ctg cag cag caa tgt tgc atg cag cta caa ggc atg atg cct cag	296
Leu Leu Gln Gln Gln Cys Cys Met Gln Leu Gln Gly Met Met Pro Gln	
70 75 80	
tgc cac tgt ggc acc agt tgc cag atg atg cag agc atg caa caa gtt	344
Cys His Cys Gly Thr Ser Cys Gln Met Met Gln Ser Met Gln Gln Val	
85 90 95	
att tgt gct gga ctc ggg cag cag cag atg atg aag atg gcg atg cag	392
Ile Cys Ala Gly Leu Gly Gln Gln Gln Met Met Lys Met Ala Met Gln	
100 105 110	
atg cca tac atg tgc aac atg gcc cct gtc aac ttc caa ctc tct tcc	440
Met Pro Tyr Met Cys Asn Met Ala Pro Val Asn Phe Gln Leu Ser Ser	
115 120 125 130	
tgt ggt tgt tgt tga tcaaacgttg gttacatgta ctctagtaat aaggtgttgc	495
Cys Gly Cys Cys *	

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gacttgc 562

<210> 21  
<211> 134  
<212> PRT  
<213> Oryza sativa

<400> 21  
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Pro Thr Leu Ala Met Gly Thr Met Asp Pro Cys Arg Gln Tyr Met Met  
35 40 45  
Gln Thr Leu Gly Met Gly Ser Ser Thr Ala Met Phe Met Ser Gln Pro  
50 55 60  
Met Ala Leu Leu Gln Gln Cys Cys Met Gln Leu Gln Gly Met Met  
65 70 75 80  
Pro Gln Cys His Cys Gly Thr Ser Cys Gln Met Met Gln Ser Met Gln  
85 90 95  
Gln Val Ile Cys Ala Gly Leu Gly Gln Gln Gln Met Met Lys Met Ala  
100 105 110  
Met Gln Met Pro Tyr Met Cys Asn Met Ala Pro Val Asn Phe Gln Leu  
115 120 125  
Ser Ser Cys Gly Cys Cys  
130

<210> 22  
<211> 45  
<212> PRT  
<213> Triticum aestivum

<400> 22  
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Arg Ala Arg Gly Ala Gln Lys Leu Cys Ala Asn Val Cys Arg Cys Lys  
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Leu Thr Ser Gly Leu Ser Cys Pro Lys Asp Phe Pro Lys  
35 40 45